PNAS Plus Significance Statements

Direct force measurements reveal that protein Tau confers short-range attractions and isoform-dependent steric stabilization to microtubules

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The microtubule-associated protein Tau is known to stabilize microtubules against depolymerization in neuronal axons, ensuring proper trafficking of organelles along microtubules in long axons. Abnormal interactions between Tau and microtubules are implicated in Alzheimer's disease and other neurodegenerative disorders. We directly measured forces between microtubules coated with Tau isoforms by synchrotron small-angle X-ray scattering of reconstituted Tau–microtubule mixtures under osmotic pressure (mimicking molecular crowding in cells). We found that select Tau isoforms fundamentally alter forces between microtubules by undergoing a conformational change on microtubule surfaces at a coverage indicative of an unusually extended Tau state. This gain of function by longer isoforms in imparting steric stabilization to microtubules is essential in preventing microtubule aggregation and loss of function in organelle trafficking. (See pp. E6416–E6425.)

Conserved interdomain linker promotes phase separation of the multivalent adaptor protein Nck

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Many eukaryotic proteins are composed of tandem arrays of modular domains, which bind peptide ligands that also often appear in tandem arrays. The interdomain linkers in such systems are often considered merely passive elements that flexibly connect the functional domains. Collective interactions among multivalent molecules lead to micron-sized phase-separated states that are thought to be important in cellular organization. Here, we show that in the multivalent signaling adaptor protein Nck, weak interactions involving an interdomain linker play a significant role in self-assembly and phase separation with ligands neuronal Wiskott-Aldrich syndrome protein (N-WASP) and phosphorylated nephrin. Our results suggest that interactions mediated by ordered modular domains may generally act synergistically with weak interactions of disordered interdomain linkers to promote phase separation. (See pp. E6426–E6435.)

Allosteric N-WASP activation by an inter-SH3 domain linker in Nck

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Actin is a monomeric protein that can polymerize into branched networks. Actin polymerization acts like an engine to drive cell movement and is regulated by multiple interacting proteins on the cell membrane. To understand the molecular details of how cells transmit signals from the membrane to the actin polymerization engine, we reconstituted this process in a test tube using seven purified proteins and membrane-coated glass beads. Using this "biomimetic" system, we discovered a sequence motif in the human protein Nck that activates a core component of the actin polymerization engine. This motif shares similarity with certain bacterial virulence factors that stimulate actin polymerization in infected human cells, suggesting that similar activation mechanisms have evolved in humans and bacterial pathogens. (See pp. E6436–E6445.)

Global shape mimicry of tRNA within a viral internal ribosome entry site mediates translational reading frame selection

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Viruses use alternate mechanisms to increase the coding capacity of their viral genomes. The dicistrovirus intergenic region internal ribosome entry site (IRES) adopts an RNA structure that can direct translation in 0 and +1 reading frames to produce the viral structural proteins and an overlapping ORFx product. Here we provide structural and biochemical evidence that the PKI domain of the IRES mimics a complete tRNA-like structure to facilitate reading frame selection and allows the viral IRES to engage the ribosome. These findings provide insight into how a viral IRES can increase the coding capacity of a viral genome. (See pp. E6446–E6455.)

Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes

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When the human genome folds up inside the cell nucleus, it is spatially partitioned into numerous loops and contact domains. How these structures form is unknown. Here, we show that data from high-resolution spatial proximity maps are consistent with a model in which a complex, including the proteins CCCTC-binding factor (CTCF) and cohesin, mediates the formation of loops by a process of extrusion. Contact domains form as a byproduct of this process. The model accurately predicts how the genome will fold, using only information about the locations at which CTCF is bound. We demonstrate the ability to reengineer loops and domains in a predictable manner by creating highly targeted mutations, some as small as a single base pair, at CTCF sites. (See pp. E6456–E6465.)

HAX-1 regulates cyclophilin-D levels and mitochondria permeability transition pore in the heart

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The massive cell death, associated with a heart attack, is mainly due to disruption of mitochondrial membrane integrity upon activation of the mitochondrial permeability transition pore. Thus, it is important to understand how this pore is regulated to prevent cardiac cell death. In this study, we reported that hematopoietic-substrate-1 associated protein X-1 (HAX-1) is an inhibitor of the pore and promotes cell survival. HAX-1 works

through recruitment of a chaperone protein called Hsp90 from cyclophilin-D, a major component of the pore. Displacement of Hsp90 from cyclophilin-D promotes cyclophilin-D degradation, resulting in inhibition of pore opening and cell death. Given that the opening of the mitochondrial permeability transition pore contributes to various diseases, our findings have broader applications reaching beyond the heart. (See pp. E6466–E6475.)

Screening for tumor suppressors: Loss of ephrin receptor A2 cooperates with oncogenic *KRas* in promoting lung adenocarcinoma

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A large number of genetic alterations have been found in the cancer genome of lung adenocarcinoma. However, they need experimental validation to determine their tumorigenic potential, as well as the therapeutic utility of individual alterations. Here, we provide an shRNA-mediated screen to validate a large set of genes for their tumor suppressor efficacy in vivo in a mouse lung adenocarcinoma model. We identified several tumor suppressors, including ephrin receptor A2 (*EphA2*) loss of which promotes adenocarcinoma in the context of oncogenic mutant *KRas* mutation. *EphA2* loss promotes cell proliferation by activating ERK MAP kinase signaling and hedgehog signaling pathways, leading to tumorigenesis. Identification of these pathways provides important therapeutic targets for lung adenocarcinoma. (See pp. E6476–E6485.)

TRPC6 channel translocation into phagosomal membrane augments phagosomal function

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Historically, pulmonary infections treated with antibiotics killed bacteria while selecting for the unintended development of pathogenic resistance. One strategy to circumvent antibiotic resistance in pulmonary infection involves targeting the host phagosome and augmenting its function. To such an end, we have identified several small molecules, (R)-roscovitine and its derivatives, which restore microbicidal activity to compromised alveolar macrophages in cystic fibrosis (CF) and enhance function in non-CF cells. The compounds utilize G protein signaling pathways that mobilize TRPC-6 channels to the plasmalemma and subsequent phagosomal membrane formation that engulfs the bacterium. The plethora of GPCRs in resident pulmonary macrophages linked to ion channel function provides a rich source for potential therapeutic approaches to macrophage-mediated disease. (See pp. E6486–E6495.)

Extremely high genetic diversity in a single tumor points to prevalence of non-Darwinian cell evolution

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A tumor comprising many cells can be compared to a natural population with many individuals. The amount of genetic diversity reflects how it has evolved and can influence its future evolution. We evaluated a single tumor by sequencing or genotyping nearly 300 regions from the tumor. When the data were analyzed by modern population genetic theory, we estimated more than 100 million coding region mutations in this unexceptional tumor. The extreme genetic diversity implies evolution under the non-Darwinian mode. In contrast, under the prevailing view of Darwinian selection, the genetic diversity would be orders of magnitude lower. Because genetic diversity accrues rapidly, a high probability of drug resistance should be heeded, even in the treatment of microscopic tumors. (See pp. E6496–E6505.)

Engineering high-affinity PD-1 variants for optimized immunotherapy and immuno-PET imaging

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Programmed cell death protein-1 (PD-1) and programmed cell death ligand-1 (PD-L1) are key targets in the treatment of cancer, but current antibody-based drugs against this pathway have inherent drawbacks that may limit their effectiveness. We used directed evolution with yeast display to engineer a nonantibody biologic based on the ectodomain of PD-1. High-affinity PD-1 was more effective than anti–PD-L1 antibodies in the treatment of mouse tumor models and could additionally be used as a PET imaging tracer to noninvasively assess the PD-L1 expression status of tumors. This engineered protein thus represents an agent useful for clinical translation and highlights the paradigm of small protein biologics for future drug development. (See pp. E6506–E6514.)

An extracatalytic function of CD45 in B cells is mediated by CD22

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Signaling through antigen receptors is essential for lymphocyte development, survival, and function. The receptor-like tyrosine phosphatase CD45 plays a critical role in these processes by regulating antigen receptor signaling via its cytosolic phosphatase domain. Despite its abundance, the function of the large, alternatively spliced extracellular domain of CD45 has remained elusive. We used CD45 transgenes either incorporating a phosphatase-inactivating point mutation or lacking the cytoplasmic domain to uncouple the enzymatic and noncatalytic functions of CD45. Both transgenes partially rescue the phenotype of CD45-deficient B cells by modulating the B cell inhibitory surface coreceptor CD22. These data demonstrate an in vivo function for the extracellular domain of CD45 in restraining CD22 inhibitory function to maintain tonic B-cell antigen receptor signaling. (See pp. E6515–E6524.)

Targeting CD146 with a ^{64}Cu-labeled antibody enables in vivo immunoPET imaging of high-grade gliomas

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Unfortunately, current practices for diagnosis and treatment of glioblastoma have failed to improve overall patient survival, which has galvanized the emergence of novel strategies based on targeting tumor-specific antigens. Herein, we show for the first time, to our knowledge, that CD146 is a promising target for noninvasive in vivo imaging and targeted therapy of glioblastoma. We developed a ^{64}Cu-radiolabeled anti-CD146 antibody

(YY146) that allowed the sensitive and specific detection of subcutaneous and orthotopic brain tumors using positron emission tomography. Additionally, YY146 showed therapeutic effects on U87MG brain cancer cells and was able to preferentially stain human resected high-grade glioma tumors. These findings indicate the clinical relevance of our antibody and its potential role in patient diagnosis, stratification, and targeted therapy. (See pp. E6525–E6534.)

Leukocyte-specific protein 1 regulates T-cell migration in rheumatoid arthritis

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We screened rheumatoid arthritis (RA)-associated copy number variations (CNVs) across the whole genome and identified significant deletion variants encompassing leukocyte-specific protein 1 (LSP1) gene. Functional assays revealed that LSP1, induced by T-cell receptor activation, negatively regulates T-cell migration. Loss of *Lsp1* promotes T-cell migration into antigen-instilled tissues and draining lymph nodes in mice with T-cell–dependent chronic inflammation. Moreover, patients with RA show diminished expression of LSP1 in peripheral T cells with increased migratory capacity. To our knowledge, our work is the first to demonstrate how CNVs result in immune dysfunction and a disease phenotype, highlighting the importance of *LSP1* CNVs and LSP1 insufficiency in the pathogenesis of RA. (See pp. E6535–E6543.)

A basal stem cell signature identifies aggressive prostate cancer phenotypes

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Aggressive cancers often possess functional and molecular traits characteristic of normal stem cells. It is unclear if aggressive phenotypes of prostate cancer molecularly resemble normal stem cells residing within the human prostate. Here, we transcriptionally profiled epithelial populations from the human prostate and show that aggressive prostate cancer is enriched for a prostate basal stem cell signature. Within prostate cancer metastases, histological subtypes had varying enrichment of the stem cell signature, with small cell neuroendocrine carcinoma being the most stem cell-like. We further found that small cell neuroendocrine carcinoma and the prostate basal stem cell share a common transcriptional program. Targeting normal stem cell transcriptional programs may provide a new strategy for treating advanced prostate cancer. (See pp. E6544–E6552.)

A 3′ untranslated region variant in *FMR1* eliminates neuronal activity-dependent translation of FMRP by disrupting binding of the RNA-binding protein HuR

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The fragile X mental retardation protein (FMRP) is most highly expressed in neurons, and is critical for proper synaptic functioning. Fragile X syndrome, a common cause of intellectual disability, is the result of absent or dysfunctional FMRP, highlighting its importance to the processes underlying learning and memory. A rapid upregulation of FMRP synthesis at the synapse in response to specific neuronal signals is a key step in maintaining a dynamic synapse, although the mechanisms governing this up-regulation are not well-understood. We show that a variant in the 3′UTR of fragile X mental retardation 1 (*FMR1*) causes the loss of this characteristic increase in synaptic FMRP synthesis, which may lead to developmental delay in patients. These data identify several mechanisms and molecules modulating activity-dependent translation of FMRP. (See pp. E6553–E6561.)

Sigma-1 receptor mediates cocaine-induced transcriptional regulation by recruiting chromatin-remodeling factors at the nuclear envelope

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The endoplasmic reticulum (ER), although functioning as protein synthesis machinery in the cell, plays other important roles that have yet to be fully unveiled. We found here that the ER can directly send an envoy protein, the sigma-1 receptor (Sig-1R), to the nuclear envelope (NE), where the Sig-1R begins to recruit chromatin-remodeling molecules through the NE integral protein emerin to control gene transcription. Thus, the Sig-1R represents a molecule that shapes the functional connection between the NE and the DNA. We also demonstrate in this study that cocaine, a Sig-1R agonist, down-regulates the critical enzyme monoamine oxidase B that influences the cocaine-induced dopamine level in a dopamine transporter-independent manner via this never-before-described, to our knowledge, Sig-1R-linked genomic action of cocaine. (See pp. E6562–E6570.)

A vacuolar phosphate transporter essential for phosphate homeostasis in *Arabidopsis*

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Phosphate is an essential nutrient for plant growth, and inorganic phosphate (Pi) is stored largely in the vacuole of plant cells. Thus, vacuolar Pi maintains homeostasis of cytosolic Pi to ensure an optimal Pi supply for plants under variable Pi status in the soil. This study uncovered in *Arabidopsis* a vacuolar phosphate transporter, VPT1, that mediates vacuolar Pi sequestration. Lack of VPT1 caused growth defects under both low-Pi and high-Pi conditions, implicating VPT1 in plant adaptation to constantly changing Pi levels in the environment. This finding not only supplies a missing link in our understanding of vacuolar Pi storage and homeostasis, but also provides a new path for engineering crops that can better adapt to variable Pi availability in the soil. (See pp. E6571–E6578.)

Circadian and feeding rhythms differentially affect rhythmic mRNA transcription and translation in mouse liver

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Rhythmic gene regulation in mouse liver results from an intertwined relationship between feeding cycles and the circadian clock. Significant efforts have been made to understand this interaction but a complete picture of the resulting diurnal transcription–translation processes is still missing. Through the simultaneous quantification of temporal transcription, accumulation, and translation of mRNA in the liver, we investigated the regulatory landscape of mice with intact or deficient circadian clock subjected to different feeding regimens. We showed that circadian clock and feeding rhythms coordinate rhythmic transcription to drive downstream rhythmic mRNA accumulation and translation. However, a subset of genes harboring 5′-Terminal Oligo Pyrimidine tract or Translation Initiator of Short 5′-UTR elements encoding proteins involved in translation and mitochondrial activity, respectively, present a transcription-independent rhythmic translation mainly regulated by feeding. (See pp. E6579–E6588.)